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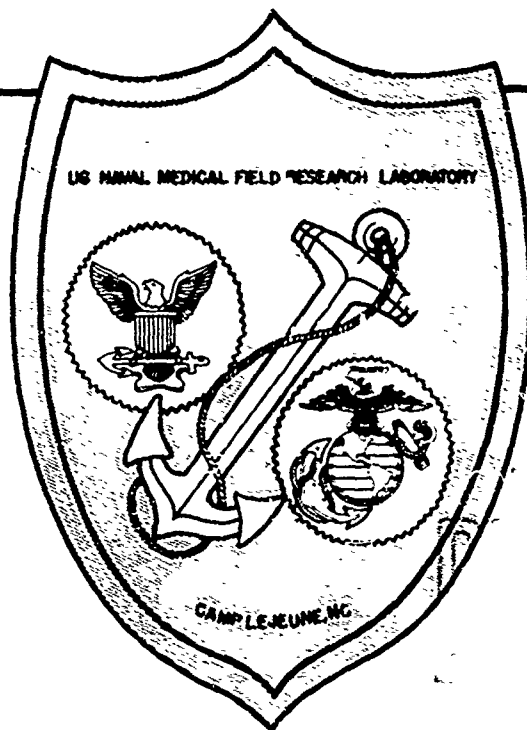
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13. ABSTRACT

Rifampin failed to inhibit adenovirus replication in tissue culture. The data suggest that rifampin would be ineffective as a chemotherapeutic agent against adenovirus disease. (U)

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Failure of Rifampin to Inhibit
Adenovirus Replication

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Rifampin failed to inhibit adenovirus replication in tissue culture. The data suggest that rifampin would be ineffective as a chemotherapeutic agent against adenovirus disease.

Rifampin is an antibiotic originally isolated from *Streptomyces mediterraneus* (8). The compound is well absorbed orally and reaches peak serum concentrations 2 to 3 hr after an oral dose (7). Rifampin has potent antibacterial and antitubercular effects, and because it enters the secretions, it has been particularly useful for the treatment of pulmonary tuberculosis and the meningococcal carrier state (1, 5).

Antiviral effects have also been demonstrated for rifampin, although the concentrations of the antibiotic required for antiviral activity are several hundred-fold greater than those producing antibacterial effects. The compound has a clear antiviral effect on a number of deoxyribonucleic acid viruses, including vaccinia, variola minor, cowpox (6, 9), African swine fever virus (3), and trachoma agent (2).

Although an inhibitory effect of rifampin on adenovirus proliferation has been suggested, the evidence to date has not been conclusive (9, 10). Rifampin would have obvious applications in controlling outbreaks of adenovirus disease among military trainees (11) if the drug inhibited adenovirus proliferation at concentrations attained in the respiratory secretions. With this in mind, we evaluated in vitro the effect of rifampin on the proliferation of adenovirus types commonly isolated from military recruits.

Adenovirus type 3 strain G.B., type 4 strain RI-67, type 7a strain S-1058, and *Herpesvirus hominis* strain Mayo 1814 were kindly provided by Sylvia Cunningham, Medical Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. All virus strains and cell culture lines were determined to be free from mycoplasma contamination. Adenovirus types and *H. hominis* virus were passed twice in primary human embryonic kidney (HEK) cells (HEM Laboratories) and at least twice in continuous human epithelium cells (HEp-2).

A 50% tissue culture infective dose (TCID$_{50}$) was calculated for the growth of the virus strains cultured with and without rifampin. For this purpose, serial dilutions ranging from $10^{-0.5}$ to 10^{-10} of adenovirus or *H. hominis* were prepared. Amounts of 0.1 ml of each virus dilution in the appropriate medium were inoculated in triplicate into HEK tubes containing rifampin at a concentration of 25, 50, or 100 µg/ml (Rifadin, lot D-1354, Dow Chemical Co.) in Eagle's minimal essential medium (MEM) or MEM alone and were incubated for up to 17 days. As an alternative, 100 TCID$_{50}$ of virus was inoculated into HEK tubes containing rifampin at a concentration of 0.390 to 200 µg/ml of MEM, and incubated for 7 days.

HEK tubes were read daily, and virus cytopathogenic effect (CPE) was estimated visually at 25 to 100%. For the calculation of each TCID$_{50}$, tissue culture tubes were considered to be infected if a virus-specific CPE affected 74% or more of the cell sheet. In addition, toxic effects of rifampin were recorded semiquantitatively as a percentage depending on the degree of rounding, granulation, and vacuolation of the cell monolayers in the presence of rifampin and MEM, but not virus.

Rifampin was toxic to HEK cells at concentrations of 25 µg/ml or greater. A rifampin con-

TABLE 1. *Adenovirus titrations in media with and without rifampin (50 µg ml)*

Test prepn	TCID₅₀ (negative logₙₑ)					
	Day 3[a]	Day 4	Day 5	Day 6	Day 7	Day 10
Adenovirus type 7 plus MEM	2.35	2.52	3.52	4.17	4.34	5.02
Adenovirus type 7 plus rifampin	2.35	3.17	3.85	4.52	4.85	>5.0
Adenovirus type 4 plus MEM	2.35	2.35	2.85	2.85	3.17	4.67
Adenovirus type 4 plus rifampin	2.35	2.85	3.35	3.67	3.85	>5.0
Adenovirus type 3 plus MEM	2.5	2.5	3.5	4.5	4.8	7.5
Adenovirus type 3 plus rifampin	1.8	2.5	3.5	4.5	4.5	6.5[b]

[a] Day after virus inoculation

[b] Chi-square test, $P > 0.50$.
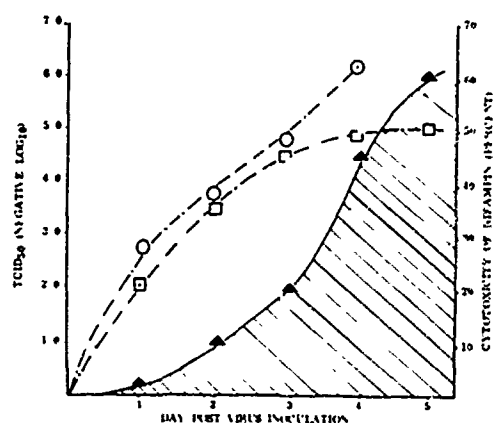
FIG. 1. *Effect of adenovirus (□), adenovirus plus rifampin (○, 100 µg/ml), and rifampin alone (▲, 100 µg/ml) on primary human embryonic kidney cells in culture.*

centration of 100 µg/ml induced 60% cytotoxicity in HEK cells by the fifth day of incubation (Fig. 1). On the seventh day of incubation, adenovirus types 3, 4, and 7 and herpes simplex virus showed 100% CPE when cultured in each two-fold dilution of rifampin from 0.39 to 200 µg/ml. No qualitative differences in the rate of development of CPE could be detected at any of these rifampin concentrations.

Results of virus titrations in media containing 50 µg of rifampin/ml are summarized in Table 1. All titrations except for adenovirus type 3 showed a higher titer of virus in the rifampin tubes than in the control tubes. The small decrease in titer of adenovirus type 3 cultured in rifampin at a concentration of 50 µg ml was not significant. In Fig. 1, comparison of effects obtained with and without rifampin (100 µg ml) indicates the enhancement of viral CPE by the cytotoxicity of the antibiotic.

In summary, we did not demonstrate an anti-

viral effect for rifampin on the proliferation of adenovirus types 3, 4, and 7, or *H. hominis.* The toxicity of high concentrations of rifampin for primary HEK cells was especially prominent after the third day of incubation (Fig. 1), and this may explain why previous workers had shown an inhibitory effect of rifampin on adenovirus plaque formation in HeLa cells. Adenovirus plaques require at least 7 days to develop (10).

Our experiments did not demonstrate that rifampin inhibited adenovirus proliferation; however, more sensitive techniques that measure the rate of synthesis of specific adenovirus precursors might have shown an inhibitory effect, particularly for adenovirus type 3. Our in vitro studies demonstrate that rifampin at 0.5 µg/ml, the peak concentration achieved in saliva after the fourth oral dose of 600 mg (4), is not likely to affect in vivo the course of an adenovirus infection. Whether rifampin would suppress in vivo an adenovirus viremia remains to be determined, because peak concentrations of 27.7 µg ml are achieved in serum after an oral dose of 900 mg of rifampin (7).

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